**Title: Statistical Shape Modeling with ShapeWorks**

**Purpose:** This SOP explains the steps, files, and programs associated with taking surfaces that have been segmented in Amira through the preparation for and execution of statistical shape modeling with ShapeWorks.

**Scope:** The methods explained in this SOP guide a user through: aligning femur surfaces, cropping femurs at a chosen landmark, converting surfaces to DICOM images, ShapeWorks preprocessing and completing a ShapeWorks run. This SOP provides step by step instructions but also attempts to explain what is happening and why during process.

**Notes:**

* Changes to this SOP should be noted and described on the final page of this document.
* Instructions are specific to a SSM project done in 2012 with femurs, but can be adapted for any dataset.
* As written, these instructions allow for creating and maintaining a separate set of files for each preprocessing step leading up to ShapeWorks. This results in a huge amount of data. If you are confident in the SSM process and want to save some space, you can overwrite previous files during every step of preprocessing, beginning with AutoCrop.

**Programs Used:**

* Amira with mesh Pack (last used version: 5.4.1)
* Notepad++ (last used version: v 5.6.8)
* Microsoft Excel 2010
* AutoCropVolumes.exe
* AutoPadVolumes.exe
* ConvertDICOM.exe
* ResampleVolumesToBeIsotropic.exe
* ShapeWorksGroom.exe
* ShapeWorksRun.exe
* ShapeWorksView.exe
* R: Statistical Program

**Prerequisite Files:**

* Femur reconstructions in .surf format.

**Procedures:**

**Crop and Align Surfaces**

1. Gather labels (.am) and surface (.surf) files for all femurs.
2. Choose a master femur to which all other femurs will be aligned and open it’s surf file in Amira. This selection can be arbitrary.
   1. dod\_n03\_R was chosen as the master.
3. For femurs that were scanned in a nearly neutral position:
   1. Open the label and surf files for one subject in Amira
   2. Attach an OrthoSlice to the labels and a SurfaceView to the surf
   3. Determine the slice # just above the lesser trochanter
      1. The lesser trochanter was chosen as a standard anatomical landmark at which we decided to crop all femurs.
      2. Record the slice #
      3. Use the Crop tool in the Properties window of the labels to crop the label at the lesser trochanter
      4. Also use the manual crop in the viewing window to crop out extraneous black space above the femur. This will greatly reduce the size of the labels file and will hasten new surface generation.
   4. In the Segmentation Editor of the cropped labels, remove segmentation from the last slice. This will ensure that upcoming surfaces will be closed.
   5. Save the cropped labels with a cropped\_ prefix
   6. Generate a new surface from the cropped labels
      1. Simplify and Smooth the surface to 30,000 faces
      2. Save the new surface as pt#\_side\_crop\_femur.surf (ascii)
   7. Align the cropped femur surface with the master femur (that has been cropped)
      1. Use manual alignment to get the femurs close together
      2. Scale Left femurs to look like Right femurs by scaling by -1 in x within the Transform Editor of the cropped surface (Absolute tab).
      3. Then attach an AlignSurfaces module and further align the surfaces
         1. Alignment type = rigid
         2. Reference surface is the master femur
         3. Turn **OFF** ‘use correspondence’
         4. Relative RMS = 0.0001
         5. Iterations = 100
   8. Apply the transform to the rotated femur using the Apply Transform button in the Transform Editor
   9. Record the final RMS error from the alignment
   10. Save the final surface as pt#\_side\_crop\_align\_femur.surf
4. For femurs that are externally rotated:
   1. Manually (roughly) align the current femur with the master (not cropped)
   2. Attach an AlignSurfaces module and align further align the current femur to the master
      1. Use the same setting for Align Surfaces as stated above
   3. Copy the transformation information from the surface to its associated labels
      1. Use the Copy button in the Transform Editor of the newly aligned femur surface
      2. Then use the Paste button in the Transform Editor of that femurs labels
   4. Apply the transform to the labels by right-clicking on them and choosing Compute 🡪 Apply Tranform
      1. Interpolation = Nearest neighbor
      2. Mode = extended
      3. Preserve = Dimensions
   5. Attach an OrthoSlice to the transformed labels and find the slice number just above the lesser trochanter
   6. Follow steps 3c-j above.

**Establish a Uniform Bounding Box**

1. Open each cropped surface and get the bounding box using the command console.
   1. The bounding box coordinates are printed to the console in this order <xmin> <xmax> <ymin> <ymax> <zmin> <zmax>.
   2. In the console, type: pt#\_side\_crop\_align\_femur.surf getBoundingBox
2. Copy the bounding box values and put them all in a text document. Once you have a document containing bounding box values for all the femurs, open the text file in Excel as space delimited. (You could use Matlab if you prefer)
3. In Excel, find the smallest min and largest max x,y, and z coordinates from the list of bounding boxes.
   1. This will tell you the coordinates of a bounding box that will encompass all the femurs.
   2. Pad the values by 10 to ensure some black space around each femur.
   3. Calculate a bounding box that is square in the xyz dimensions (i.e. length in x direction = length in y direction = length in y direction). Having square (aka isotropic) data will be easier to deal with in other programs.
   4. Try to pad black space somewhat evenly on each side of the femurs.
   5. Make sure that your chosen bounding box is, in fact, larger than all the femurs
4. In Amira,
   1. Open any image set (I used Amira’s example lobus.am images)
   2. Set the bounding box of the images to the values you found in the previous step. Use the command console to set the bounding box (command: lobus.am setBoundingBox xmin xmax ymin ymax zmin zmax).
      1. I used -51 74 -70 55 -10 115 for the final set of femurs used in the 2012 femur paper.
   3. Resample the images to 512 x 512 x whatever will create isotropic voxel spacing.
      1. Do this by attaching a Resample module to the images.
      2. I chose 512 x 512 those values because they maintained good resolution but limited subsequent DICOM images to ~100MB/set.

**Convert to DICOM Images**

1. Attach a ScanConvertSurface to the surface that has been cropped and aligned to the master.
2. Assign the Field of ScanConvertSurface to the image stack that has the desired dimensions and bounding box.
3. Click Apply
   1. The result should be a label field with the dimensions and bounding box you specified.
4. Save the label field in DICOM format. Use a separate subfolder for each femur.

NOTE: Instructions for the next steps assume that you are running them on a Unix system. Windows executables are also available and may be useful if the Unix binaries are not compiled for your specific system. For Windows, the process will be very similar – just change slashes from / to \ and add .exe.

**Convert from DICOM to NRRD**

1. Begin with DICOM files for each subject
   1. For JOR 2012 paper they are located at /usr/sci/projects/hip/SSM\_Project/FemurSSM\_2012\_paper/ and then under DOD\_controls/scanconverted\_volumes and similar folders for IHC\_cam, NIH\_cam, NIH\_controls
2. Edit parameter file DICOMToNRRD.xml
   1. If the DICOM files are isotropic (or at least equally spaced in xy) then it shouldn’t matter if you have the swapXY flag is on or not.
   2. Change the inputs to point to each of the directories where the DICOM files are located.
      1. Ex. ./DOD\_controls/scanconverted\_volumes/dod\_n03\_R
      2. Ex. ./IHC\_cam\_patients/scanconverted\_volumes/IHC05\_R
   3. Change the outputs so all NRRDs will be located in one directory
      1. Ex. ./nrrd\_volumes/dod\_n03\_R.nrrd
3. Run the ConvertDICOM executable
   1. From the FemurSSM\_2012\_paper/ folder: **Executables/Tools/ConvertDICOM paramfiles/DICOMToNRRD.xml**

**AutoCrop by Centering and Trimming Bounding Boxes**

**NOTE: this step seems redundant since I previously aligned all the surfaces and they are fairly well centered in the bounding box. It also seems a bit strange that there is this centering and trimming step followed by a padding step, especially since I do those things myself in Amira. – Talk with developers to see if this is a needed step.**

1. NOTE: This step will save a lot of computational processing time by removing excess space around the femurs. This step basically centers the bounding boxes on the femurs.
2. Edit the parameter file called AutoCrop.xml
   1. Make sure input files are coming from the nrrd\_volumes/ folder.
   2. Set <centervolume> to 1
   3. Set <foregroundvalue> to 1. This is an intensity value (for us it is 1 because our data only have femur = 1 and background = 0). The AutoCrop will then search for the largest connected component in each direction associated with this intensity value. It does this for each femur (starting at the lower left corner of each) and finds the smallest necessary dimensions for the entire group. This is kind of similar to what was done manually in Amira to choose a uniform bounding box.
3. Run the AutoCropVolumes executable
   1. From the FemurSSM\_2012\_paper/ folder: **Executables/Tools/AutoCropVolumes paramfiles/AutoCrop.xml**
4. This will create new NRRD files in a cropped folder

**Resample to Truncate the Voxel Spacing to 2 Decimal Places**

1. NOTE: ShapeWorksRun will only consider the first two decimal places. There may be small differences in voxel spacing beyond two decimal places; this step just makes sure that those discrepancies are eliminated and the voxels are really the same size. This will eliminate the “out of bounds” errors that we used to get in past versions of ShapeWorks.
2. Edit the parameter file ResampleNRRD.xml
   1. Make sure inputs are coming from the cropped/ folder
      1. Ex. ./cropped/dod\_n03\_R.nrrd
   2. Make sure outputs are put in a resampled/ folder
   3. Check the iso spacing value to be sure it is correct.
      1. The <isoSpacing> value is based on the voxel spacing of the DICOM image sets created by ScanConvertSurface in Amira.
3. Run the ResampleVolumesToBeIsotropic executable
   1. **Executables/Tools/ResampleVolumesToBeIsotropic paramfiles/ResampleNRRD.xml**

**Pad Volumes to make sure thay are all the same Size (this step is redundant for our femurs but may be useful for other data)**

1. Edit the AutoPad.xml parameter file.
   1. Make sure input files are from the resampled/ folder
   2. Make sure outputs are put in a padded/ folder
2. Run the AutoPadVolumes executable: **Executables/Tools/AutoPadVolumes paramfiles/AutoPad.xml**

**Isolate femur from background and fill holes (this step is redundant for our femur sets)**

1. Edit the parameter file preprocess1.xml
   1. Make sure inputs are coming from the padded/ folder
      1. Ex. ./padded/dod\_n03\_R.nrrd
   2. Make sure outputs are put in a preprocessed/ folder
      1. Ex. ./preprocessed/dod\_n03\_R.nrrd
   3. Make sure <background> = 0, and <foreground> = 1
   4. The <pad> value is redundant and could probably be removed from the parameter file.
   5. **What is the transform\_file?**
2. Run the ShapeWorksGroom executable with keywords isolate and hole\_fill
   1. **Executables/ShapeWorks/ShapeWorksGroom paramfiles/preprocess1.xml isolate hole\_fill**

**Antialiasing, fast march distance transforms, smoothing**

1. Edit the parameter file preprocess2.xml
   1. Change input to the preprocessed/ directory
      1. Ex. ./preprocessed dod\_n03\_R.nrrd
   2. Change the output to the same preprocessed/ directory
      1. Add a ‘D’ to end of each output file to indicate that it has had the distance transform calculated.
      2. Ex ./preprocessed/dod\_n03\_RD.nrrd
   3. Change the sigma and iterations values if advised
2. Because this step can take a very long time, we have split up the femur groups and run this step for each group in parallel
   1. E.g. preprocess2DOD.xml
3. Run the ShapeWorksGroom executable with antialias fastmarching and blur keywords
   1. **Executables/ShapeWorks/ShapeWorksGroom paramfiles/preprocess2DOD.xml antialias fastmarching blur &**
   2. **Executables/ShapeWorks/ShapeWorksGroom paramfiles/preprocess2IHC.xml antialias fastmarching blur &**

**Initialize Particle Correspondences with Hierarchal Splitting method in ShapeWorksRun**

1. Edit the parameter file called correspondenceInitCP.xml
   1. NOTE: This does the initial particle placement and splitting but not optimization. This also does not consider or remove scaling (i.e. no Procrustes). This does allow for an optimization “cutting” plane that limits particle placement only to regions above the plane.
      1. The optimization plane is on when <adaptivity\_mode> is set to 3.
      2. The cutting planes are specified as a clockwise listing of three points on the plane (left hand coordinate system). For the femurs the cutting plane is the same for every femur to eliminate shape variability caused by alignment after surface cropping at the lesser trochanter.
      3. The actual coordinates for the plane can be found by choosing a slice number and then using Seg3D to choose three points on that slice and recording the world coordinates of those points.
         1. In Seg3D change the main view to the axis you want. Then click on the Globe icon in the bottom right corner to change from index numbers to physical coordinates. Choose and three points (in clockwise order) in the plane (i.e. slice) that you want.
         2. An arbitrary plane can also be chosen by using landmarks in your data and choosing three points based on your landmarks.
   2. There must be a cutting plane designated for every dataset (i.e. every femur)
   3. Set the number of particles to 2048
   4. Set the <processing mode> to 1. This allows initialization but stops before optimization.
2. Run ShapeWorksRun with correspondenceInitCP.xml
   1. **Executables/ShapeWorks/ShapeWorksRun paramfiles/correspondenceInitCP.xml**
   2. This will not change the NRRD files. It creates point files for the particles and puts them in a folder called initCP/

**Optimize Particle Correspondences using Entropy Minimization**

1. Edit the parameter file called correspondenceFinalCP.xml
   1. Make sure the <processing\_mode> is -2. This is the optimization mode.
   2. Check the point file inputs to make sure they come from the point files you just made in the previous step.
   3. Still need to give it cutting plane(s).
   4. Check the added optimization parameters
      1. Starting and ending regularization: This ensures that the shape matrix is regularized at all times (i.e. all the eigenvalues are real and nonzero). This is part of the entropy minimization scheme. The regularization values are proportional to the entropy in the system.
      2. Procrustes interval and scaling: Interval is the span of iterations after which you want realignment. Setting the scaling value to 1 computes modular scale.
      3. Recompute regularization interval: After how many intervals do you want to recompute the regularization coefficient.
2. Run ShapeWorksRun corespondenceFinalCP.xml
   1. **Executables/ShapeWorks/ShapeWorksRun paramfiles/correspondenceFinalCP.xml**
   2. This will produce the final point files for each femur and put them in a folder called finalCP.

**Analyze Your Final Data Using ShapeWorksView (must be done on Windows)**

1. Download the final point files from the finalCP/ folder
2. Edit the parameter file called analyze.xml to make sure it points to the finalCP/ folder where the point files are located. Also, make sure that the correct femurs are identified as being in either group 1 (control) or group 2 (patients)
3. Make sure you have the ShapeWorksView.exe executable on your local machine.
4. Open a command window and navigate to the directory containing the project files (e.f. Executables, paramfiles, finalCP)
5. Run the ShapeWorksView executable
   1. **Executables\ShapeWorks\ShapeWorksView.exe paramfiles\analyze.xml**
6. You can output any shape you are looking at in VTK format by choosing File 🡪WriteSurfaceMesh
7. These VTK files can be viewed using ParaView.
   1. Paraview can write the files out in STL format. After opening the file and clicking on Analyze, click on the Filter menu and choose the Triangulate filter.
   2. To give the meshes a smoother appearance use the Generate Surface Normals filter with the feature angle set to 60°.
      1. IMPORTANT NOTE: If you want to compare shapes in phantomthick you will need to flip some normal. If you want to display the control mean shape with protrusions of the patient mean shape (or different mode shapes) as positive values, check the Flip Normals option in the Generate Surface Normals filter when creating the stl files for *both* the control and the patient shapes.
      2. If you want to look at a specific patient you may need to play around with the direction of the normal to get the color plot values (+ or -) to be what you want.
   3. Save the data in binary STL format
      1. If you want to be able to opne the STL in PreView then you will need to save in ASCII format, which results in larger sized files.
   4. The stl files can be opened in Amira and saved in ASCII .surf format for use with phantomthick.
   5. NOTE: PCA is done entirely in the ShapeWorksView. There is some eigen decomposition that takes place in the Optimization but that is purely for entropy balancing.

**Statistical Analysis**

1. PCA and Visualization is done entirely in ShapeWorksView
2. Generate Mode Data:
   1. Use the Compute ModeLengths.exe and the analyze.xml file
   2. Executables\ShapeWorks\ComputeModeLengths.exe paramfiles\analyze.xml modeinfo.txt
      1. The modeinfo.txt file will contain numerical information about the modes (eigenvalues?). Perhaps most important is the column called titled ‘PV’ which gives information about the cumulative percent variation captured by the modes.
      2. Open the modeinfo.txt file in Excel and plot the PV column. Also note in this column how many modes it takes to capture 80%, 90%, etc of the variation among the shapes.
3. Parallel Analysis to Determine the Significant Number of Modes
   1. NOTE: See the article “Parallel Analysis: a Method for Determining Significant principal Components” by Franklin (1995) *J Vegetation Science*.
   2. You need the statistical package called R. (<http://cran.r-project.org>)
   3. In R, change the directory to a working directory you want.
   4. Make sure you have the file ParallelAnalysis.R
      1. Open this file in Notepad++ and edit the fPrefix
      2. Make sure that DIMS equals #particles\*3
      3. Use the comments to help you.
   5. In the R command window type: source ('HotellingT2.R')
   6. The output is a PDF file (PA.pdf) with the scree plot. The number of significant modes is determined by counting the number of black circles before the green sequence (simulation using random modes) crosses the black sequence (using real mode values from data)
4. Hotelling T2 test for group Discrimination
   1. This test determines whether differences between the group mean shapes are significant.
   2. Make sure you have the file HotellingT2.R
      1. Modify the value for variable 'e' on line40 to indicate the number of modes to use (these are chosen via parallel analysis)
      2. Modify other values hard-coded (e.g. input mode info filename and location on line34) in the R code to suit the data.
      3. The output is a CSV file (HotellingT2Profile.csv) with the p-value and value of the test statistic.

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| **Revision Date** | **Revision Author** | **Revision Description** |
| 7/30/12 | M. Harris | Added changes to include AutoCrop of the bounding box and the optimization cutoff plane. |
| 7/31/12 | M. Harris | Added all steps used for the 2012 femur SSM project. Some are redundant but can be useful for future projects. |
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